SUPPLEMENTAL MATERIALS AND METHODS

Plasmids

The multiple cloning sites of all plasmids were replaced by FseI (5') and AscI (3') (FA) restriction sites. For transient expression pCS2 derived vectors were used. To generate pCS2-FKBP-linker-FA (N-terminal FKBP-linker tag) the FKBP sequence of pC_4EN-F1 (ARIAD) was amplified using oligos 5'-CGGGATCCATGGGAGTGCAGGTGGAAA-3' and 5'-TTAGGCCGGCCATTGTTATTTTCCAGTTTTAGAAGCTCCA-3' and then BamHI/FseI cloned into pCS2-Myc_6-FA (replaces Myc_6 tag) yielding pCS2-FKBP-FA. In a second step oligos 5'-GCTCCCGCCCCTGCCGCTGCCGCCCCTGCCCCCGTGGCCGCTGCCGCCCCTG CCGCTGCCGCCGGCCGG-3' and 5'-CCGGCGGCAGCGGCAGGGGCGGCAGCGGCCACGGGGGCAGGGGCGGCAGCG GCAGGGGCGGGAGCCCGG-3' were phosphorylated, annealed and ligated into FseI-cut pCS2-FKBP-FA yielding pCS2-FKBP-linker-FA (Linker peptide sequence: NNNGRAPAPAAAAPAPVAAAAPAAAA). To generate the corresponding vectors for stable integration into human cells *FKBP-linker* was amplified using oligos 5'-ATACCAACCGGATGGATGGGAGTGCAGGTGGAAAC-3' and 5'-AGAGGCGCGCCCTCGAGAGG-3' and BstXI/AscI cloned into FseI/AscI-cut pcDNAL/FRT/TO-FA and pcDNA5/loxP/TO-FA yielding pcDNAL/FRT/TO-FKBP-linker-FA and pcDNA5/loxP/TO-FKBP-linker-FA, respectively. pCS2-FA-FRB (C-terminal FRB tag) was generated by amplifying the sequence of the FRB domain of mTOR from pC_4-RHE (ARIAD) using oligos 5'-GGCGCGCCATCCTCTGGCATGAGATGTG-3' and 5'-

GCTCTAGATCACTTTGAGATTCGTCGGAACAC-3' followed by Asc1/Xba1-cloning of this fragment into pCS2-FA-His-Flag-His-Flag (*FRB* replaces His-Flag-His-Flag tag). The respective vectors for stable integration were generated as follows: *FRB* was amplified with oligos 5'-TCAGGCCGGCCGTTTAAACG-3' and 5'-ATAACGCGTTCACTTTGAGATTCGTCGGAACACAT-3' and then FseI/MluI cloned into FseI/AscI-cut pcDNAL/FRT/TO-FA and pcDNA5/loxP/TO-FA to yield pcDNAL/FRT/TO-FA-FRB and pcDNA5/loxP/TO-FA-FRB, respectively.

Cloning of *SCC1* and *SMC3* has been described elsewhere (Stemmann *et al*, 2001; Schöckel *et al*, 2011). *SCC1* (with stop codon) was subcloned into pCS2-FKBP-linker-FA and pcDNAL/FRT/TO-FKBP-linker-FA as well as (without stop codon) into pCS2-FA-FRB and pcDNAL/FRT/TO-FA-FRB via FseI/AscI restriction sites. These sites were also used to subclone *SMC3* into pCS2-FA-FRB and pcDNA5/loxP/TO-FA-FRB. The open reading frame of *SMC1A* was amplified from human thymus cDNA (Clontech) using oligos 5'-ATAGGCCGGCCCATGGGGTTCCTGAAACTGATTG-3' and 5'-GGCGCGCCCTACTGCTCATTGGGGTTGG-3' and FseI/AscI cloned into pCS2-FKBP-linker-FA.

The position of the internal hinge domain FKBP/FRB tags in the peptide sequence of Smc1 and Smc3 were chosen based on existing structural information and previous yeast study (Haering *et al*, 2002; 2004; Gruber *et al*, 2006). *FKBP* was inserted into Smc1 following Glu583 and FRB was inserted into Smc3 following Glu602 (human annotation), both nested into short linker peptides of 5 (N-terminal) and 9 amino acids (C-terminal). To generate internally tagged Smc1, we first inserted NheI/SphI restriction sites via classical mutagenesis PCRs using oligos 5'-ATAGGCCGGCCCATGGGGTTCCTGAAACTGATTG-3', 5'-

GTTTGCATGCGCCGCTAGCCTCATCTGTAGGCTTCACCTCC-3', 5'-GATGAGGCTAGCGGCGCATGCAAACTCCGGGAGCTGAAGGG-3' and 5'-GGCGCGCCCTACTGCTCATTGGGGTTGG-3'. This product was then FseI/AscI cloned into pCS2-FA. Finally, *FKBP-linker* (only partial linker) was amplified using oligos 5'-ATAGGCTAGCGCAGGATCCATGGGAGTGCAGGT-3' and 5'-TTCAGCATGCGGGAGCCCGGCCATTGTTATTTTC-3' and then NheI/SphI cloned into the open reading frame of Smc1 yielding pCS2-Smc1-int. FKBP. To generate internally tagged Smc3, we first inserted NheI/XmaI restriction sites via classical mutagenesis PCRs using oligos 5'-AATGGCCGGCCCATGTACATAAAGCAGGTGATTAT-3', 5'-TGGTCCCGGGGCCGCTAGCTTCAGGATAGGCTGTATCCC-3', 5'-CCTGAAGCTAGCGGCCCCGGGACCAATGATGCTATTCCTATGATC-3' and 5'-AATGGCGCGCCTTAACCATGTGTGGTATCATC-3'. This product was then FseI/AscI cloned into pCS2-FA. Finally, *FRB* was amplified using oligos 5'-ATAGGCTAGCGCAGGATCCATGATCCTCTGGCA-3' and 5'-TTACTACCCGGGAGCCCGGCCGTTGTTGTTCT-3' and then NheI/XmaI cloned into the open reading frame of Smc3 yielding pCS2-Smc3-int. FRB. For the generation of stable cell lines *Smc3-int. FRB* was FseI/AscI subcloned into pcDNAL/FRT/TO-FA.

Since the target site of the siRNA used for endogenous Smc1 knockdown was situated in the open reading frame of its gene, we had to make our transgenes RNAi resistant by mutating the target site from GGAAGAAAGTAGAGACAGA to GAAAGAAGGTGGAAACGGA. This was done by classical mutagenesis PCRs using oligos 5'-ATGGAGCTCCTGTGGGCAAG-3', 5'-CCTCCGTTTCCACCTTCTTTCGTTCTTCCAGATCCAGACGG-3', 5'-AACGAAAGAAGGTGGAAACGGAGGCCAAGATCAAGCAAAAGCTG-3' and 5'-

TTGGCTAGCTCACTCTCCAGC-3' (FKBP-linker-Smc1) or 5'-CATGGATCCTGCGCTAGCC-3' (Smc1-int. FKBP). The resulting fragment was then SacI/NheI (FKBP-linker-Smc1) or SacI/BamHI (Smc1-int. FKBP) cloned into the open reading frame of Smc1 to generate "Smc1 (siResist)" and "Smc1-int. FKBP (siResist)". For the generation of stable cell lines the two RNAi resistant transgenes were FseI/AscI subcloned into pcDNA5/loxP/TO-FKBP-linker-FA (Smc1 (siResist)) and pcDNA5/loxP/TO-FA (Smc1-int. FKBP (siResist)), respectively.

To create an Scc1-Smc1 (siResist) fusion construct, where Scc1 and Smc1 (siResist) are linked by a short peptide linker (N-WTRSRP-C), *Smc1 (siResist)* together with a linker sequence was amplified from pCS2-FKBP-linker-Smc1 (siResist) using oligos 5'-ATACTCGAGATAACAATGGCCGGGCTCCCGCCC-3' and 5'-GGCGCGCCCTACTGCTCATTGGGGTTGG-3'. This fragment was then via XhoI/AscI fused in frame to the 3' end of Scc1 in pcDNAL/FRT/TO-Scc1-FRB.

The Histone 2B-FRB fusion construct was generated by FseI/AscI subcloning of Histone 2B ORF into pCS2-FA-FRB. To generate the FKBP expression construct the FKBP ORF was amplified with oligos 5'-CGGGATCCATGGGAGTGCAGGTGGAAA-3' and 5'-TTAGGCGCGCCTTATTCCAGTTTTAGAAGCTCCA-3' and cloned into pCS2-Myc_6-FA via BamHI/AscI restriction (replacing Myc_6 tag).

SUPPLEMENTAL FIGURES

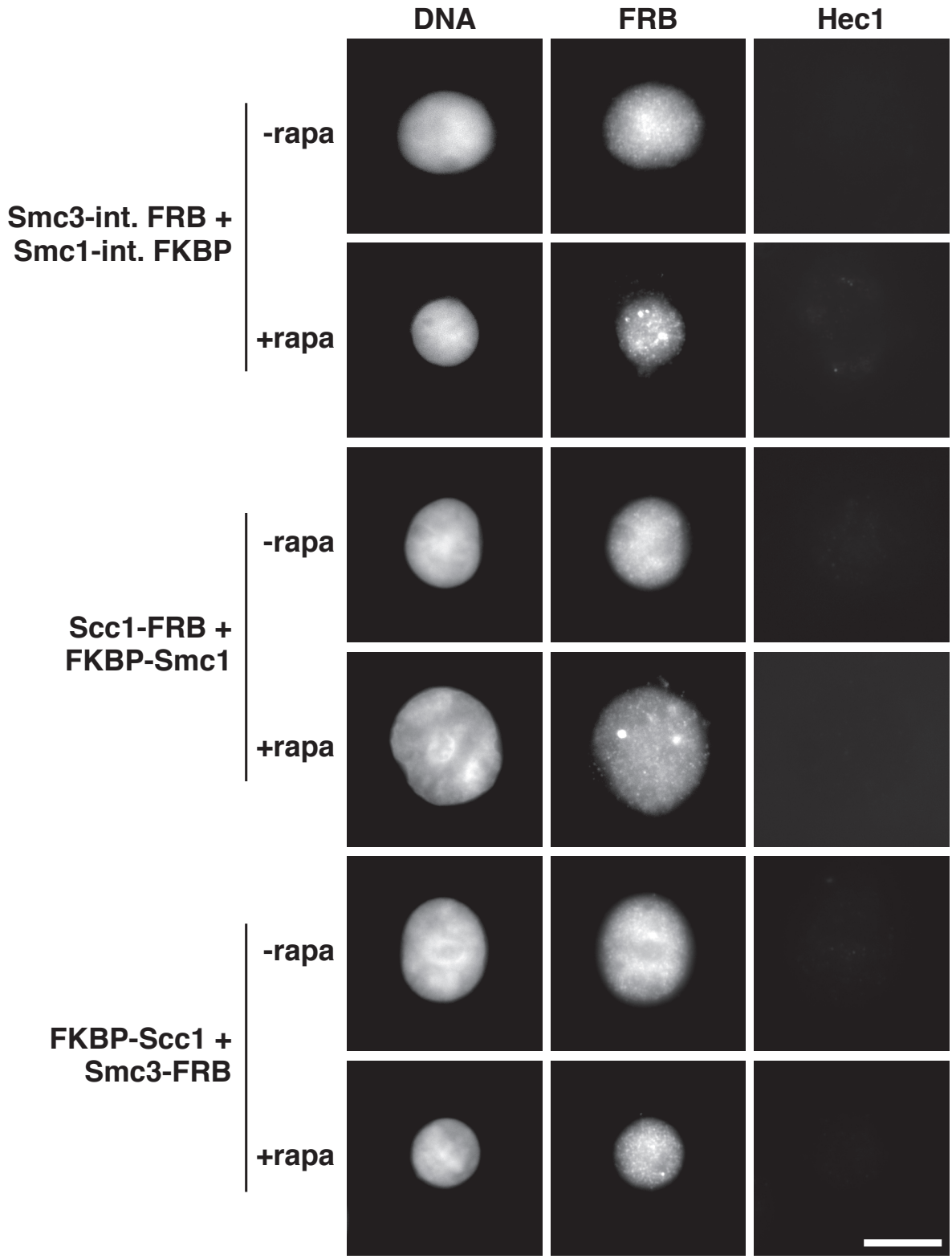

Figure S1 Transgenic cohesin subunits are loaded onto chromatin before mitotic entry. Depicted are exemplary interphase cells from the experiments shown in Figure 4C-F that had not yet entered mitosis. This demonstrates that the designed cohesin complexes were associated with interphase chromatin. Scale bar = 10 μm.

SUPPLEMENTAL REFERENCES

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